Behavioral and Physiological Features of Chickens Diversely Selected for Resistance to Avian Disease. 1. Selected Inbred Lines Differ in Behavioral and Physical Responses to Social Stress

R. Dennis,* H. M. Zhang,† L. D. Bacon,† I. Estevez,* and H. W. Cheng‡,[1]

*Department of Animal and Avian Sciences, University of Maryland, College Park, Maryland 20742; †Avian Disease and Oncology Laboratory, USDA-ARS, East Lansing, Michigan 48823; and ‡Livestock Behavior Research Unit, USDA-ARS and Animal Sciences Department, Purdue University, West Lafayette, Indiana 47907

ABSTRACT To test the hypothesis that genetic variations in response to social stress modulate susceptibility to disease in poultry, aggressive behaviors induced by social stress were measured in chickens of different inbred lines selected for disease resistance (line 6_3) or susceptibility (lines 7_2 and $15I_5$), as well as 2 recombinant congenic strains (B and X). At 15 wk of age, roosters from each genetic line or strain were randomly assigned to pairs for intraline male-male aggression tests (n = 8 per line). Based on the results of the intraline aggression tests, the roosters were divided into 2 groups, winners and losers. At 16 wk of age, the roosters were randomly paired as winners vs. winners and losers vs. losers for interline aggression tests, i.e., line 6_3 vs. 7_2 and $15I_5$; line 7_3 vs. line $15I_5$; and strain X vs. strain B. Similarly, at 17 wk of age, line 6_3 vs. strains X and B, and line 7_2 vs. strains X and B were tested. The tests were conducted in a novel cage that was similar to their home cages, to provide a neutral space for both roosters being tested. Each pair was videotaped for 15 min. Male-male interaction-induced aggressive behaviors were markedly different among the genetic lines. Compared with roosters of lines $15I_5$ and 7_2, line 6_3 roosters generally showed fewer aggressive behaviors, including aggressive pecks and fights, as well as durations ($P < 0.05$). Roosters of the recombinant congenic strains X and B, each possessing a unique random 87.5% genome of line 6_3, exhibited low aggressive behaviors, which were similar or equal to the level of line 6_3 in both intraline and interline aggression tests ($P = 0.05$). These results may indicate that some of the gene(s) commonly carried between strains X and B as well as line 6_3 likely played an important role in governing their lower levels of aggression. The present chicken lines may be used as animal models for investigation of the cellular mechanisms of genetic-environmental interactions on disease resistance and stress responses.

(*Key words*: genetic selection, aggression, behavior, physical index, chicken)

2004 Poultry Science 83:1489–1496

INTRODUCTION

Chronic social stress in chickens reared in high-density environments, such as a battery cage system, may be a major problem in the modern poultry industry, because social stress has a large impact on the chickens' welfare and performance. Inability of chickens to adapt to their social environment results in a greater susceptibility to disease (Gross and Siegel, 1988; Awadalla, 1998) and increases the frequency of hostile behaviors, such as cannibalism, aggression, and feather pecking (Mauldin, 1992; Via, 1999; El-Lethey et al., 2000). One solution to these problems may be through genetic selection for greater resistance to pathogen infections associated with less aggressive behavior resulting from a reduction or limitation of social stress.

Several chicken lines have been genetically selected for differences in antibody responses to a variety of pathogens or vaccine challenges such as *Salmonella typhimurium* (Al-Murrani et al., 2002), *Salmonella pullorum* (Pevzner et al., 1981b), Marek's disease (Pevzner et al., 1981a), and *Escherichia coli* (Pitcovski et al., 2001). These studies have shown that disease resistance in chickens, like many other economically important traits in farm animals, may be attributed to genetic and environmental components. Social stress resulting from aggression, feather pecking, and cannibalism is one of the most important environmental components affecting disease susceptibility in chickens

Received for publication November 12, 2003.
Accepted for publication March 30, 2004.
[1]To whom correspondence should be addressed: hwcheng@purdue.edu.

Abbreviation Key: ADOL = Avian Disease and Oncology Laboratory, East Lansing, MI; Lines 6_3, 7_2, and $15I_5$ = inbred lines selected for disease resistance or susceptibility; RCS = recombinant congenic strain; Strains B and X = 6C.7B and 6C.7X, recombinant congenic strains between lines 6_3 and 7_2.

(Gross and Siegel, 1985). An understanding of the association of disease resistance and adaptability to social stress is critical in preventing pathogen infections and harmful behaviors associated with welfare problems in poultry.

Three inbred lines of White Leghorn chickens have been selected for resistance (line 6_3) or susceptibility (lines 7_2 and $15I_5$) to viral-induced tumors, i.e., Marek's disease and lymphoid leucosis, at the Avian Disease and Oncology Laboratory (ADOL).[2] The original selection was started in 1939 from 10 distinct White Leghorn strains. In 1940, line 6_3 was developed by mating between males of strains 1, 2, 5, and 7 and females of strains 2 and 3; line 7_2 was from males of strains 1 and 7 and females of strains 2 and 7; line $15I_5$ was from males of strains 2 and 7 and females of strains 2 and 3. The genetic characteristics of each line have recently been reported (Bacon et al., 2000; Bacon, 2002). Although the cellular mechanism underlying the differences in disease resistance among the lines is still under evaluation, previous studies have indicated that resistance may be related to line differences in expression of genes that control the neuroendocrine system and immunity, and line differences in genotype-environmental interactions.

Crittenden (1993) reported that inheritance of resistance to avian leukosis viruses can be simple, e.g., lack of a viral receptor determined by one gene. In contrast, Albers (1993) indicated that resistance to most diseases, including Marek's disease, has a low heritability, and defense against infectious diseases often requires resource allocation (Gross et al., 2002). In addition, disease resistance could be related to selection-induced changes in immunity.

Previous studies have found that, compared with line 7_2, line 6_3 had a low concentration of serum IgG and low responses of lymphoid cells to T cell mitogens (Bacon, 2002; Yonash et al., 2002). Furthermore, the disease resistance differences between the genetic lines could be related to selection-induced changes in the stress buffering systems, such as the hypothalamus-pituitary-adrenal axis, which controls an animal's ability to adapt to stressors, including alterations in behaviors and immunity (Cheng et al., 2001a,b). Inadequate coping to social stress increases antisocial behaviors (Craig and Muir, 1996) and susceptibility to pathogen infection (Sheridan et al., 2000). Social-related behaviors including cannibalism and aggressive pecking have been used as stress indicators (Savory and Mann, 1997; Via, 1999; El-Lethey et al., 2000) to evaluate chicken welfare (Hughes, 1991; Duncan, 1998; Cloutier and Newberry, 2000) and to examine behavior-management interaction (Mauldin, 1992; Newberry et al., 2002).

Collectively, genetic selection for disease resistance has created chicken lines with significantly different phenotypes. Each line has unique characteristics in basic immune parameters and resistance to avian disease. These differences in disease resistance may be associated with selection-induced changes in stress responses, such as behavioral and physiological variations in response to social stress. However, this hypothesis has not been evaluated. The objective of the present study was to examine the effect of genetic selection for tumor resistance on behavioral traits and physical parameters, especially internal and external aggressive behaviors, i.e., exhibition of aggression when paired with roosters of the same line or among different lines.

[2]Avian Disease and Oncology Laboratory, USDA-ARS, East Lansing, MI.

MATERIALS AND METHODS

Genetic Lines

Three inbred lines, 6_3, 7_2, and $15I_5$, and 2 recombinant congenic strains (RCS) 6C.7B (strain B) and 6C.7X (strain X) were examined in the current study. The 6C.7 recombinant congenic strains B and X are representatives of 19 RCS resulting from a crossing of lines 6_3 and 7_2, followed by 2 backcrosses to the parental line 6_3 and then breeding inter se within each of the RCS. Strains B and X carry a unique random 87.5 and 12.5% genome from lines 6_3 and 7_2, respectively. Line and strain differences in resistance to avian disease (Bacon et al., 2000, 2001) and physiological characteristics (Bacon and Palmquist, 2002; Yonash et al., 2002) have been reported previously.

Chicks of both sexes from each line were intermingled in colony cages and reared under the same conditions at the ADOL until about 8 wk of age. At 8 wk of age, male chickens were transported approximately 500 miles to the Purdue Poultry Farm (Purdue University, Indiana). To avoid any errors caused by disease or other unexpected factors, at least 20 roosters per line were shipped to ensure there were enough roosters to be tested based on the experimental design. Upon receipt, chickens from each line and strain were randomly assigned to individual cages with 2,000 cm^2 of floor space. Each chicken was provided with 2 nipple drinkers and fed ad libitum with a standard rooster diet containing 12.1% CP and 3,167 kcal of ME/kg. A light schedule of 9L:15D was followed daily except for testing days. To avoid effects of transport stress and allow for adaptation to the new environment, behavioral tests began when the roosters were 15 wk old.

Social Stress

Previous studies have reported that social stress can be induced in animals, including chickens, by mixing unfamiliar individuals from different cages, strains, or both (Noble et al., 1993; Gvaryahu et al., 1996; Cheng et al., 2002). Based on these findings, male-male interactions in internal- and external-line pairs were used as a social stressor.

Behavioral Tests

Internal (roosters paired within the same line) and external (roosters paired from different lines) aggressive behaviors of each genetic line were tested in the present study. There were 3 trials, in which each rooster was tested only once per trial with approximately 1 wk of rest for the rooster to recover before the next test. During the tests, the chickens' behavior, physical fitness, and any injuries were observed closely via computer monitors.

Chickens were cared for in strict accordance with the rules and regulations of the Federation of Animal Science Societies (Craig et al., 1999). The experimental protocol was approved by the institutional Animal Care and Use Committee at Purdue University.

Trial 1: Intraline Aggression Test. At 15 wk of age, paired roosters of the same line were placed into a novel cage that was similar to their home cages, which minimized the fear response to a new environment and provided a neutral space for both roosters being tested. Eight pairs of roosters from each of the 5 lines and strains (6_3, 7_2, $15I_5$, X, and B) were videotaped for 15 min each; videos were analyzed for aggressive behavior. Roosters were marked differently in blue on the tail feathers such that both roosters displayed the same amount of marking, but were easily differentiated by the observer (Shea et al., 1990). Roosters were introduced simultaneously to the cage from doors on either end of the cage. Observations started immediately after the introduction of the pair of roosters in the cage.

The video recordings were analyzed using The Observer software from Noldus.[3] To limit errors from the observer, the same persons conducted observations throughout the study.

During a 15-min exposure time per pair, aggressive behaviors were recorded using a modification of methods used by Estevez et al. (2002) as follows: 1) fight with peck (one rooster delivers one or more kicks to another accompanied by at least one strong peck); 2) fight (one or both roosters deliver at least one kick to another, but no pecks); 3) peck to head (one rooster delivers peck(s) to the head or neck of another rooster); and 4) peck to body (one rooster delivers peck to the body or legs of another rooster). Pecks were counted in bouts. Each bout was recorded as the time in seconds, from the first peck until pecking has ceased and the aggressor raised its head or the 2 birds separated. The duration of each bout was recorded to reduce a potential bias against the more rigorously aggressive roosters, as each individual peck can be indiscernible to an observer during highly aggressive displays. The identity of the roosters either giving or receiving the interaction, and winner or loser was also recorded within each pair.

Trial 2: Interline Aggression Test between Genetic Lines. Based on the results of trial 1, roosters from each line were divided into 2 groups, winners and losers. At 16 wk of age, roosters were randomly chosen from each group of each line and paired (n = 8 per group) as winners vs. winners and losers vs. losers to avoid any bias from mixing winners vs. losers. There were 8 pairs for each of the 4 combinations: 6_3 vs. 7_2, 6_3 vs. $15I_5$, 7_2 vs. $15I_5$, and X vs. B. Paired roosters were marked, placed in novel cages, and their behavior observed as described for trial 1.

Trial 3: Interline Aggression Test between Genetic Lines and their RCS. At 17 wk of age, roosters were randomly chosen from each line and paired as winners vs. winners and losers vs. losers, as determined in trial 1. There were 8 pairs of each of the following 4 combinations: 6_3 vs. X, 6_3 vs. B, 7_2 vs. X, and 7_2 vs. B. Testing procedures were identical to trials 1 and 2.

Body and Organ Weights

Body and organ weights of the roosters were collected at 18 wk of age. The BW was taken immediately after removing the roosters from their cages. Roosters were humanely killed by cervical dislocation. The right adrenal gland, spleen, liver, and heart were dissected without fat. Organ weights were measured using a microanalytical scale with a resolution of 0.01 mg,[4] and was recorded as absolute (g) and relative weight. Each relative organ weight was presented as a ratio of the absolute organ weight (mg or g) to BW (kg).

Statistical Analysis

Data were analyzed using SAS software (SAS Institute, 1992). Behavioral data of the intraline aggression tests were analyzed by one-way ANOVA for the effects of line on aggressive behaviors. Means reported were least squares means. Logarithmic transformation was used for tests of significance to meet the assumption of normality (an arbitrary constant was added to each observation to ensure that all data points of zero were included in the analysis). To correct for variance heterogeneity, the residual variances were considered separately for each line (variance partitioning). Contrasts were formulated to compare the results of the intraline tests in predetermined groups and adjusted within groups using the Sidak correction for a priori pairwise comparisons to maintain an experimental alpha of 0.05 (Westfall and Young, 1993). Contrasts were limited to 3 groups to decrease the risk of type I errors. The predetermined groups were as follows: group 1 compared the behaviors exhibited among inbred lines 7_2, 6_3, and $15I_5$, group 2 compared the strains X and B with line 6_3, and group 3 compared the strains X and B with line 7_2. Nonparametric analysis using permutation was used for the test of significance in interline test for the effects of line and previous status (winner or loser) on aggressive behaviors and possible interactions. Body weight and relative organ weight data were analyzed by a one-way ANOVA meeting all of the required assumptions. Tukey-Kramer adjustment[5] was used in computations of the t-values for all possible pairwise comparisons to maintain an overall experimental alpha of 0.05 (Kramer, 1956). Statistical significance was at $P < 0.05$.

[3]Noldus Information Technology Inc., Leesburg, VA.
[4]Analytical Plus, OHAUS, Brooklyn, NY.
[5]Excel, Microsoft Corporation, Redmond, WA.

TABLE 1. Aggressive behaviors (mean + SEM) of paired roosters from same genetic line

Strain[1]	Fight with peck Number	Fight with peck Duration (s)	Total pecks Number	Total pecks Duration (s)
$15I_5$	4 ± 1.2^b	22 ± 4.1^b	10 ± 3.1^b	18 ± 2.6^b
7_2	3 ± 1.0^{ab}	26 ± 5.7^b	7 ± 2.2^{ab}	18 ± 4.9^b
6_3	1 ± 0.6^{ac}	6 ± 2.7^{ac}	3 ± 0.9^a	5 ± 2.3^a
B	0 ± 0.1^c	3 ± 2.5^c	2 ± 0.5^a	14 ± 3.6^a
X	0 ± 0.1^c	9 ± 8.7^c	1 ± 0.8^a	9 ± 4.3^a

[a–c]Means within a column lacking a common superscript differ significantly ($P < 0.05$) (8 pairs/line).

[1]Lines 6_3, 7_2, and $15I_5$ = inbred lines selected for disease resistance or susceptibility; strains B and X = 2 recombinant congenic strains between lines 6_3 and 7_2.

RESULTS

Aggressive Behavioral Tests in Paired Roosters from the Same Genetic Line

Phenotypic variation in aggressive behaviors was observed in roosters among the $15I_5$, 6_3, and 7_2 lines. Behavioral variations were reflected in the number and average duration of fights with peck, total number of pecks, and the average duration of pecks (Table 1, $P < 0.05$). Among the lines, $15I_5$ roosters showed the most aggression toward each other in duration and total number of instances, whereas line 6_3 roosters exhibited the least total aggression.

Compared with roosters of line 6_3, line $15I_5$ roosters in the intraline testing exhibited greater numbers, longer average duration of fights with pecks (Table 1, $P < 0.05$). and a higher total number of pecks (pecks to head, pecks to body, and fight with pecks, $P < 0.05$) with a longer average duration of pecks. Roosters of line 7_2 showed a significantly longer average duration of total pecks compared with line 6_3 roosters ($P < 0.05$). No differences in aggressive pecks and duration were found between roosters of lines $15I_5$ and 7_2 in the intraline test ($P > 0.05$).

Compared with strains X and B, line 7_2 roosters exhibited more fights, resulting in greater numbers and longer average durations of fight with pecks to each other than roosters from either strain X or B (Table 1, $P < 0.05$). No differences in aggressive behavior were found between roosters of line 6_3 and strains X or B when they were paired within line ($P > 0.05$).

Aggressive Behavioral Tests in Paired Roosters from Different Lines

The same aggressive order, $15I_5 \geq 7_2 > 6_3$, was confirmed in the mixed line aggressive behavioral tests (Table 2). There was no interaction between line and previous status (winner or loser, $P > 0.05$). Previous status from intraline test had no effect on aggression in the interline test ($P > 0.05$). In the 82 pairs for the interline aggression test, 5 roosters were removed with minor injuries to their heads. These roosters were from lines 6_3 (2) and 7_2 (3) after being paired with $15I_5$ roosters. Pecks delivered by $15I_5$ roosters were notably harder in intensity than those delivered by most roosters of other lines.

When paired with line 6_3 roosters, line $15I_5$ roosters initiated more fights with pecks (Table 2, $P < 0.05$), and delivered a higher number of total pecks ($P < 0.05$) during the 15-min trials than roosters of line 6_3. Moreover, line $15I_5$ roosters exhibited a greater duration of fights with pecks compared with line 6_3 roosters ($P < 0.05$). When paired with line 7_2 roosters, line $15I_5$ roosters had a higher average frequency and duration of total pecks ($P < 0.05$, respectively) and duration of fights with pecks ($P < 0.05$). In the pairings between roosters of lines 7_2 and 6_3, line 6_3 roosters received more fights with pecks and total pecks from line 7_2 roosters than they delivered ($P < 0.05$, respectively).

When roosters from lines 7_2 or 6_3 were paired with roosters from their RCS strains, X or B, the roosters of line 7_2 but not line 6_3 delivered more pecks to the head ($P < 0.05$) with a higher frequency of total pecks ($P < 0.05$) to their counterparts from both strains B and X (Table 3).

TABLE 2. Aggressive behaviors (mean ± SEM) of roosters paired from different genetic lines

Mixed pair	Line[1]	Peck to head Number	Peck to head Duration[2] (s)	Total pecks Number	Total pecks Duration (s)	Fight with peck Numbers	Fight with peck Duration (s)
$15I_5$ and 7_2	$15I_5$	8 ± 5.0	6 ± 2.9	14 ± 8.5	22 ± 4.9*	4 ± 2.3	13 ± 3.7*
	7_2	3 ± 5.0	3 ± 2.9	6 ± 8.5	9 ± 4.9*	1 ± 2.3	3 ± 3.7*
$15I_5$ and 6_3	$15I_5$	16 ± 6.9	8 ± 2.7	29 ± 11.9*	25 ± 10.7	6 ± 2.2*	11 ± 3.1*
	6_3	1 ± 6.9	3 ± 2.7	2 ± 11.9*	13 ± 10.7	0 ± 2.2*	3 ± 3.1*
7_2 and 6_3	7_2	5 ± 2.3	5 ± 2.7	12 ± 4.7*	17 ± 7.6	3 ± 1.5*	7 ± 3.9
	6_3	2 ± 2.3	6 ± 2.7	2 ± 2.7*	7 ± 7.6	0 ± 1.5*	1 ± 3.9

[1]Lines 6_3, 7_2, and $15I_5$ = inbred lines selected for disease resistance or susceptibility.

[2]Average duration = total time spent observed behavior/number of behaviors observed.

*Significant difference ($P < 0.05$) with each combination (8 pairs/combination).

TABLE 3. Aggressive behaviors (mean + SEM) of roosters paired between line 6_3 or 7_2 and recombinant congenic strains B or X

Mixed pair	Line[1]	Peck to head		Total peck		Fight with peck	
		Number	Duration[2] (s)	Number	Duration (s)	Number	Duration (s)
7_2 and X	7_2	8 ± 2.0*	6 ± 1.1*	11 ± 3.9*	12 ± 3.5*	0.3 ± 0.21	2.9 ± 2.0
	X	1 ± 2.0*	1 ± 1.1*	1 ± 3.9*	2 ± 3.5*	0.1 ± 0.21	0.3 ± 2.0
7_2 and B	7_2	4 ± 1.1*	4 ± 1.2*	6 ± 1.8*	6 ± 1.8*	0.3 ± 0.13	0.6 ± 0.4
	B	0 ± 1.1*	1 ± 1.2*	0 ± 1.8*	2 ± 1.8*	0.1 ± 0.13	0.2 ± 0.4
6_3 and X	6_3	4 ± 2.1	10 ± 3.9	8 ± 3.2	15 ± 4.5	0.3 ± 0.13	0.6 ± 0.2
	X	1 ± 2.1	1 ± 3.9	3 ± 3.2	3 ± 4.5	0.1 ± 0.13	0.4 ± 0.2
6_3 and B	6_3	3 ± 1.8	2 ± 1.9	5 ± 2.6	4 ± 2.7	NE	NE
	B	2 ± 1.8	3 ± 1.9	4 ± 2.6	6 ± 2.7	NE	NE
B and X	B	4 ± 2.1	9 ± 4.8	6 ± 5.0	17 ± 6.3*	0.6 ± 0.32	2.4 ± 1.4
	X	2 ± 2.1	1 ± 4.8	7 ± 5.0	2 ± 6.3*	0.0 ± 0.32	0.0 ± 1.4

[1]Line 6_3, and 7_2 = inbred lines selected for disease resistance or susceptibility; strains B and X = 2 recombinant congenic strains between lines 6_3 and 7_2.

[2]Average duration = total time spent observed behavior/number of behaviors observed.

[3]NE = no fighting.

*Significant difference ($P < 0.05$) with each combination (8 pairs/combination).

Line 7_2 roosters also displayed longer average durations of pecks to the head ($P < 0.05$) and total pecks ($P < 0.05$) to roosters of strains B and X when paired in the same cages. Line 6_3 roosters exhibited longer average durations of total pecks ($P < 0.05$) but not numbers of pecks than their paired counterparts from strain X but not strain B ($P < 0.05$). Strain B roosters exhibited a longer average duration of total pecks but not peck numbers than their strain X partners in the same pairs ($P < 0.05$). There were no differences observed in aggressive behavior in the mixed pairs of line 6_3 and strain B roosters ($P > 0.05$).

Results of the winners (winners were the ones who chased paired partners and delivered more aggressive pecks with longer durations) during male-male interactions are presented in Table 4. During the 8 replications, roosters of line $15I_5$ won more frequently over lines 7_2 (6 to 2) and 6_3 (7 to 1). Line 7_2 won more often over line 6_3 (6 to 2), strain B (7 to 1), and strain X (8 to 0). Line 6_3 won over strain X (5 to 2) but was equal to strain B. The strain B roosters won 4 of 6 contests against strain X roosters. The line winner order was $15I_5 > 7_2 > 6_3 = B > X$.

TABLE 4. Number of wins out of 8 pairs of one genetic line roosters paired with other line roosters within novel cages

Line or strain[1]	Wins	Line/ strain[2]	Wins	No aggression observed
$15I_5$	7	6_3	1	0
$15I_5$	6	7_2	2	0
7_2	6	6_3	2	0
7_2	7	B	1	0
7_2	8	X	0	0
6_3	4	B	4	0
6_3	5	X	2	1
B	4	X	2	2

[1]Line 6_3, 7_2, and $15I_5$ = inbred lines selected for disease resistance or susceptibility; strains B and X = 2 recombinant congenic strains between lines 6_3 and 7_2.

[2]Roosters were paired from the line within the same rows (8 pairs/ per combination).

Body Weight and Organ Weights

Body weights were compared across all genetic lines and strains, and strain B was lighter than all other lines and strain X (Table 5, $P < 0.05$). There were no differences in BW among the other 3 genetic lines and strain X ($P > 0.05$).

Roosters of line 7_2 had heavier relative adrenal glands and hearts than those of all other lines and strains (Table 5, $P < 0.05$, respectively). The relative spleen weights were also greater in line 7_2 roosters than those of line $15I_5$, line 6_3, and strain B ($P < 0.05$), but not strain X ($P > 0.05$). In addition, roosters of line 7_2 had heavier relative livers than those of line $15I_5$ ($P < 0.05$), but equivalent to those of line 6_3, strains B and X ($P > 0.05$). The strain X roosters had heavier relative livers than line 6_3 roosters ($P < 0.05$).

DISCUSSION

The Effects of Selection on Social Stress Responses and Well-Being

Inbred chicken lines 6_3, 7_2, and $15I_5$ were selected for resistance or susceptibility to Marek's disease and lymphoid leukosis under a constant infection pressure (review, Bacon, 2002). Strains X and B were developed by crossing line 6_3 with its counterpartner (line 7_2), and each strain carries a unique random 87.5% genome from line 6_3. A previous study has demonstrated that, compared with lines 7_2 and $15I_5$, line 6_3 has a greater resistance to avian viral-induced tumors (Bacon, 2002). The present study further demonstrated there were line differences in aggressive behaviors, in frequency of occurrence and duration, and in response to a social stress (male-male interaction). Compared with roosters of line $15I_5$ and line 7_2, line 6_3 roosters had a lower frequency and duration of aggressive behaviors, including aggressive pecks and fights, in intraline (paired within the same line) and interline tests (paired with roosters from lines $15I_5$ or 7_2).

TABLE 5. Body weight and relative organ weights (mean + SEM) differences between roosters of the genetic lines

Line or strain[1]	BW (kg)	Adrenal gland (mg/kg of BW)	Heart (g/kg of BW)	Liver (g/kg of BW)	Spleen (g/kg of BW)
$15I_5$	1.75 ± 0.028[a]	63 ± 2.9[a]	5.2 ± 0.36[a]	14.3 ± 0.86[a]	1.26 ± 0.048[a]
7_2	1.75 ± 0.028[a]	77 ± 2.9[b]	6.8 ± 0.36[b]	16.4 ± 0.86[a]	1.47 ± 0.048[b]
6_3	1.72 ± 0.028[a]	57 ± 2.9[a]	4.5 ± 0.36[a]	14.8 ± 0.86[ab]	1.16 ± 0.048[a]
B	1.60 ± 0.028[b]	64 ± 2.9[a]	5.1 ± 0.36[a]	16.1 ± 0.86[abc]	1.27 ± 0.048[a]
X	1.71 ± 0.028[a]	66 ± 2.9[a]	4.8 ± 0.36[a]	17.2 ± 0.86[c]	1.30 ± 0.048[ab]

[a-c]Means within a column lacking a common superscript differ significantly ($P < 0.05$).

[1]Lines 6_3, 7_2, and $15I_5$ = inbred lines selected for disease resistance or susceptibility; strains B and X = recombinant congenic strains between lines 6_3 and 7_2.

The genetic basis of the differences in social stress-induced aggressive behaviors among the lines may be reflected in different behavioral strategies for coping with environmental challenges. The less aggressive behaviors of line 6_3 may be beneficial in avoiding harmful damage in fighting and in maintaining a pecking order, resulting in a calmer environment. Consistent with the hypothesis, previous studies have shown that, within a group, following development of peck order or social system, a stable social group can be maintained (Mench and Ottinger, 1991), which provides a less stressful environment (Gross and Siegel, 1985). In contrast, social encounters with frequent fights could be physiologically and psychologically stressful for roosters from lines $15I_5$ and 7_2, which could be a cost to a bird aggressively maintaining its high social status. Similar to the current findings, previous studies have shown that when paired kind-and-gentle hens with aggressive counterpartners were maintained in groups, the kind-and-gentle hens had less stress reaction, less cannibalism, and fewer aggressive pecks (Craig and Muir, 1996; Cheng et al., 2002). The present and previous findings suggest that, in chickens, there exist different coping methods to environmental challenges, which is reflected in the chickens' well-being.

Present and previous studies indicated a positive correlation between stress response and susceptibility to disease (Gross and Siegel, 1988; Awadalla, 1998; Quan et al., 2001). Sheridan et al. (2000) reported that heritable resistance to disease was associated with the variation in response to social stress. Stress-induced corticosterone secretion has been used as an indicator of stress reactions in selection of chickens for genetic improvement in health and well-being (Gross and Siegel, 1985). In Gross and Siegel's (1985) study, chickens from a line with greater stress-induced corticosterone response were especially susceptible to viral infection. Animals (including chickens) showing more aggression, such as winners in short-term encounters, have increased levels of corticosterone (Barnard et al., 1993; Knapp and Moore, 1995; Cheng et al., 2002; Veenema et al., 2003). Alterations of stress response systems, such as the hypothalamus-pituitary-adrenal and the sympathetic-medullary-adrenal axes, are thought to be a final common pathway to control animal behavior-immunity interactions in response to stressors (Siegel, 1995), which, in turn, affect immunity. Stress-induced immunodepression, including inhibition of cell-mediated and humoral immunities have been found in chickens (Gross and Siegel, 1988; Dohms and Metz, 1991; Plytycz and Seljelid, 2002), which could be reflected in disease resistance. Taken together, behavioral responses to stress are genetically associated with immune functions, and animals with inadequate behavioral strategies in response to stressors could be at high risk for susceptibility to disease, which may be a cost for these animals to maintain high social status (Barnard et al., 1993).

Selection-Related Changes in Physical and Physiological Characteristics

Previous studies have shown that aggression and social dominance in chickens are influenced by their physical characteristics, such as BW (Cloutier and Newberry, 2000). Such an influence is unlikely in the present study, because there were no significant differences in the BW of roosters among the lines and strain X, although strain B was smaller than the other genetic groups. Although the mechanisms underlying the different regulation of aggressive behaviors among the present chicken lines and the RCS remains unclear, previous studies have shown that selection in animals, including chickens, based on one characteristic, could affect gene(s) or system(s) that are associated with behavioral and physiological characteristics that are unique to each line in response to social stress. Consistent with the hypothesis, Cheng et al. (2001a,b; 2002) reported that chickens divergently selected for high or low productivity and survivability resulting from cannibalism and aggression had different expressions of neurotransmitters, hormones, and immunity. The differential changes of these systems potentially affected each line's ability to cope with various stressors.

Changes in immune system status, such as development of the spleen and bursa of Fabricius, phagocytic activity, antibody production, and T lymphocyte subpopulations were found in turkeys selected for increased 16-wk BW (Li et al., 2000, 2001). Similarly, Yonash et al. (2002) and Bacon and Palmquist (2002) used the same lines as those in the current study and reported that there were differential immune parameters, such as IgG concentrations, levels of interferon, and response of lymphoid cells to T cell mitogens among the lines. The observation here of a larger spleen and bursa in line 7_2 compared with line 6_3 is consistent with a previous report

(Lee et al., 1981). Based on present and previous observations, the selection-related differences in behavioral patterns and immune characteristics could be viewed as evidence that coping strategies of the selected breeds are due to inheritance or phenotypic variation associated with behavioral, physiological, and neuroendocrine characteristics. The results of this investigation suggest that genetic selection based on genotypes or phenotypes of disease resistance might, directly or indirectly, have modified the corresponding genetic components that govern chickens' physiological characteristics and behavioral pattern in response to social stressors, such as male-male social interactions.

Genetic Components Regulating Stress Response

Data from RCS strains X and B further supported the hypothesis that social stress-induced differences in regulations of behavioral patterns are gene-dependent. The 87.5% of the genome that strains X and B each possess is a randomly assorted sample of genes from line 6_3, and the other 12.5% is a randomly assorted sample of genes from line 7_2 (Bacon, 2002). In the intra- and interline aggression tests, roosters from strains B and X exhibited the least aggression, which was at a level similar or equal to the levels of line 6_3. These results could indicate that the gene(s) commonly carried between strains X and B, as well as line 6_3, may be related to their kind-and-gentle behaviors. However, there was evidence that strain X tends to act with less aggression than strain B. If this is confirmed, the present RCS strains may offer an important opportunity to identify the gene responsible for aggressiveness, as there are only limited genetic differences between strains B and X. Furthermore, it is of interest that chickens of strain B have higher levels of IgG than strain X (Yonash et al., 2002).

In conclusion, the present investigation demonstrated that there were line differences in the aggressive behaviors in response to social environmental challenge. Those differences might be associated with each line's unique characteristics in disease resistance. These results indicated that selection for disease resistance in chickens may have altered their coping capability to stress. The present chicken lines may be used as models for investigation of the molecular and cellular mechanisms of genetic-environmental interactions on disease resistance and stress responses.

ACKNOWLEDGMENTS

The authors thank R. Kulkarni and D. Ferguson for producing and preparing the shipment of chickens from the ARS ADOL in East Lansing, and S. Wilcox of the Livestock Behavior Research Unit for helping with collection of data. The authors also thank Don Lay of the Livestock Behavior Research Unit, USDA-ARS for his assistance in preparing the manuscript, and thank L. Douglas of the University of Maryland for assistance with the statistical analysis.

REFERENCES

Albers, G. A. A. 1993. Breeding for disease resistance: Fact and fiction. Arch. Geflugelk. 57:56–58.

Al-Murrani, W. K., I. K. Al-Rawi, and N. M. Raof. 2002. Genetic resistance to *Salmonella typhimurium* in two lines of chickens selected as resistant and sensitive on the basis of heterophil/lymphocyte ratio. Br. Poult. Sci. 43:501–507.

Awadalla, S. F. 1998. Effect of some stressors on pathogenicity of *Eimeria tenella* in broiler chicken. J. Egypt. Soc. Parasitol. 28:683–690.

Bacon, L. D. 2002. The National Registry of Genetically Unique Animal Populations: USDA-ADOL Chicken Genetic Lines. National Animal Germplasm Program, East Lansing, MI.

Bacon, L. D., H. D. Hunt, and H. H. Cheng. 2000. A review of the development of chicken lines to resolve genes determining resistance to diseases. Poult. Sci. 79:1082–1093.

Bacon, L. D., H. D. Hunt, and H. H. Cheng. 2001. Genetic resistance to Marek's disease. Curr. Top. Microbiol. Immunol. 255:121–141.

Bacon, L. D., and D. Palmquist. 2002. Chicken lines differ in production of interferon-like activity by peripheral white blood cells stimulated with phytohemagglutinin. Poult. Sci. 81:1629–1636.

Barnard, C. J., J. M. Behnke, and J. Sewell. 1993. Social behaviour, stress and susceptibility to infection in house mice (*Mus musculus*): Effects of duration of grouping and aggressive behaviour prior to infection on susceptibility to *Babesia microti*. Parasitology 107:183–192.

Cheng, H. W., G. Dillworth, P. Singleton, Y. Chen, and W. M. Muir. 2001a. Effect of genetic selection for productivity and longevity on blood concentrations of serotonin, catecholamines, and corticosterone of laying hens. Poult. Sci. 80:1278–1285.

Cheng, H. W., S. D. Eicher, Y. Chen, P. Singleton, and W. M. Muir. 2001b. Effect of genetic selection for group productivity and longevity on immunological and hematological parameters of chickens. Poult. Sci. 80:1079–1086.

Cheng H. W., P. Singleton, and W. M. Muir. 2002. Social stress in laying hens: Differential dopamine and corticosterone responses after intermingling different genetic strains of chickens. Poult. Sci. 81:1265–1272.

Cloutier, S., and R. C. Newberry. 2000. Recent social experience, body weight and initial patterns of attack predict the social status attained by unfamiliar hens in a new group. Behavior 137:705–726.

Craig, J. V., W. F. Dean, G. B. Havenstein, K. K. Kruger, K. E. Nestor, G. H. Purchase, P. B. Siegel, and G. L. van Wicklen. 1999. Guidelines for poultry husbandry. Pages 55–66 in Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. Federation of Animal Science Societies, Savoy, IL.

Craig, J. V., and W. M. Muir. 1996. Group selection for adaptation to multiple-hen cages: Beak-related mortality, feathering, and body weight responses. Poult. Sci. 75:294–302.

Crittenden, L. B. 1993. New approaches to genetic resistance in poultry. Arch. Geflugelkd. 57:59–68.

Dohms, J. E., and A. Metz. 1991. Stress—Mechanisms of immunosuppression. Vet. Immunol. Immunopathol. 30:89–109.

Duncan, I. J. 1998. Behavior and behavioral needs. Poult. Sci. 77:1766–1772.

El-Lethey, H., V. Aerni, T. W. Jungi, and B. Wechsler. 2000. Stress and feather pecking in laying hens in relation to housing conditions. Br. Poult. Sci. 41:22–28.

Estevez, I., R. C. Newberry, and L. J. Keeling. 2002. Dynamics of aggression in the domestic fowl. Appl. Anim. Behav. Sci. 76:307–325.

Gross, W. B., and P. B. Siegel. 1985. Selective breeding of chickens for corticosterone response to social stress. Poult. Sci. 64:2230–2233.

Gross, W. B., and P. B. Siegel. 1988. Environment-genetic influences on immunocompetence. J. Anim. Sci. 66:2091–2094.

Gross, W. B., P. B. Siegel, and E. W. Pierson. 2002. Effects of genetic selection for high or low antibody response on resistance to a variety of disease challenges and the relationship of resource allocation. Avian Dis. 46:1007–1010.

Gvaryahu, G., U. Shalev, B. Robinzon, and N. Snapir. 1996. Intermingling heavy and light strain chickens may cause social stress. Poult. Sci. 75:849–851.

Hughes, B. O. 1991. Behavioural needs and poultry welfare. Vet. Rec. 128:286.

Knapp, R., and M. C. Moore. 1995. Hormonal responses to aggression vary in different types of agonistic encounters in male tree lizards, *Urosaurus ornatus*. Horm. Behav. 29:85–105.

Kramer, C. Y. 1956. Extension of multiple range tests to group means with unequal numbers of replications. Biometrics 12:307–310.

Lee, L. F., P. C. Powell, M. Rennie, L. J. N. Ros, and L. N. Payne. 1981. Nature of genetic resistance to Marek's disease in chickens. J. Natl. Cancer Inst. 66:789–796.

Li, Z., K. E. Nestor, Y. M. Saif, J. W. Anderson, and R. A. Patterson. 2001. Effect of selection for increased body weight in turkeys on lymphoid organ weights, phagocytosis, and antibody responses to fowl cholera and Newcastle disease-inactivated vaccines. Poult. Sci. 80:689–694.

Li, Z., K. E. Nestor, Y. M. Saif, and M. Luhtala. 2000. Flow cytometric analysis of T lymphocyte subpopulations in large-bodied turkey lines and a randombred control population. Poult. Sci. 79:219–223.

Mauldin, J. M. 1992. Application of behavior to poultry management. Poult. Sci. 71:634–642.

Mench, J. A., and M. A. Ottinger. 1991. Behavioral and hormonal correlates of social dominance in stable and disrupted groups of male domestic fowl. Horm. Behav. 25:112–122.

Newberry, R. C., S. Cloutier, and C. M. Ulibarri. 2002. Effects of group size and stock density on cannibalism in laying hens. Poult. Sci. 81(Suppl. 1):34. (Abstr.)

Noble, D. O., E. A. Dunnington, and P. B. Siegel. 1993. Ingestive behavior and growth when chicks from lines differing in feed consumption are reared separately or intermingled. Appl. Anim. Behav. Sci. 35:359–368.

Pevzner, I. Y., I. Kujdych, and A. W. Nordskog. 1981a. Immune response and disease resistance in chickens. II. Marek's disease and immune response to GAT. Poult. Sci. 60:927–932.

Pevzner, I. Y., H. A. Stone, and A. W. Nordskog. 1981b. Immune response and disease resistance in chickens. I. Selection for high and low titer to *Salmonella pullorum* antigen. Poult. Sci. 60:920–926.

Pitcovski, J., A. Cahaner, E. D. Heller, T. Zouri, B. Gutter, Y. Gotfried, and G. Leitner. 2001. Immune response and resistance to infectious bursal disease virus of chicken lines selected for high or low antibody response to *Escherichia coli*. Poult. Sci. 80:879–884.

Plytycz, B., and R. Seljelid. 2002. Stress and immunity: Minireview. Folia Biol. 50:181–189.

Quan, N., R. Avitsur, J. L. Stark, L. He, M. Shah, M. Caligiuri, D. A. Padgett, P. T. Marucha, and J. F. Sheridan. 2001. Social stress increases the susceptibility to endotoxic shock. J. Neuroimmunol. 115:36–45.

SAS Institute. 1992. SAS User's Guide to the Statistical Analysis System. SAS Institute Inc., Cary, NC.

Savory, C. J., and J. S. Mann. 1997. Behavioural development in groups of pen-housed pullets in relation to genetic strain, age and food form. Br. Poult. Sci. 38:38–47.

Shea, M. M., J. A. Mench, and O. P. Thomas. 1990. The effect of dietary tryptophan on aggressive behavior in developing and mature broiler breeder males. Poult. Sci. 69:1664–1669.

Sheridan, J. F., J. L. Stark, R. Avitsur, and D. A. Padgett. 2000. Social disruption, immunity, and susceptibility to viral infection. Role of glucocorticoid insensitivity and NGF. Ann. N. Y. Acad. Sci. 917:894–905.

Siegel, H. S. 1995. Stress, strains, and resistance. Br. Poult. Sci. 36:3–22.

Veenema, A. H., O. C. Meijer, E. R. de Kloet, J. M. Koolhaas, and B. G. Bohus. 2003. Differences in basal and stress-induced HPA regulation of wild house mice selected for high and low aggression. Horm. Behav. 43:197–204.

Via, S. 1999. Cannibalism facilitates the use of a novel environment in the flour beetle, *Tribolium castaneum*. Heredity 82:267–275.

Westfall, P. H., and S. S. Young. 1993. Resampling-Based Multiple Testing: Examples and Methods for P-value Adjustment. J Wiley, New York.

Yonash, N., L. D. Bacon, and E. J. Smith. 2002. Concentration of immunoglobulin G in plasma varies among 6C.7 recombinant congenic strains of chickens. Poult. Sci. 81:1104–1108.